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Patentanmeldung Nr. Patent application No. Demande de brevet n°

03021447.2

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For the President of the European Patent Office

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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
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Compositions for the treatment and prevention of diabetes mellitus

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Compositions for the treatment and prevention of diabetes mellitus

The present invention relates to compositions for the treatment or prevention of diabetes mellitus, or other conditions associated with impaired glucose tolerance such as syndrome X and obesity. More specifically, the present invention relates to compositions comprising comprising a catechin found in green tea (hereinafter: EGCG) particularly (-) epigallocatechin gallate, and a peroxisome proliferator-activated receptor gamma (hereinafter: PPARg) agonist. In another aspect, the present invention relates to the use of EGCG in the manufacture of a nutraceutical composition for concomitant consumption in the treatment or prevention of diabetes or obesity by administration of a PPARg agonist. In still another aspect, the invention relates to a method of treatment or prevention of diabetes mellitus, or other conditions associated with impaired glucose tolerance such as syndrome X and obesity wherein an effective amount of a composition comprising EGCG and a PPARg agonist is administered to an individum in need of such treatment.

The term composition as used herein comprises a mixture of EGCG and a PPARg agonist; a pharmaceutical formulation containing EGCG and a PPARg agonist together with conventional pharmaceutical excipients and auxiliaries; and nutraceutical compositions such as food and beverages containing one or both of these active ingradients. The term nutraceutical as used herein denotes a usefulness in both the nutritional and pharmaceutical field of application. Thus, the novel nutraceutical compositions can find use as supplement to food and beverages, and as pharmaceutical formulations for enteral or parenteral application which may be solid formulations such as capsules or tablets, or liquid formulations, such as solutions or suspensions.

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The term "EGCG" as used herein comprises (-)-epigallocatechin gallate (EGCG in the narrower sense) and/or one or more derivatives (esterified forms, glycosides, sulphates) thereof, or other catechins found in green tea such as (-) epigallocatechin (EGC), (-) epicatechin-3-gallate (ECG), (-) epicatechin (EC), (+) gallocatechin, and (+) catechin and derivatives thereof. Of primary interest for use in the present invention is (-)-epigallocatechin gallate.

The term "PPARg" agonist as used herein denotes a compound which activates or modulates the nuclear receptor PPARg, thereby enhancing insulin sensitivity. PPARg agonist may be a full agonist, a partial agonists or selective PPAR modulator/agonist, or PPAR combination agonist.

Examples of such PPARg agonists are thiazolidinediones (hereinafter: TZD's), such as Glitazones, e.g., ciglitazone, rosiglitazone and pioglitazone; natural occuring PPARg agonists, e.g. ligustilide and other phthalide ananalogues as disclosed in European patent application No. 3010804.7 the contents of which is incorporated herein for reference purposes, e.g., (E)-senkyunolide E; senkyunolide C; senkyunolide B; 3-butyl-4,5,6,7-tetrahydro-3,6,7-trihydroxy-1(3H)-isobenzofuranone; 3-butyl-1(3H)-isobenzofuranone; 3-butylphthalide; 3-butylidenephthalide; chuangxinol; ligustilidiol; senkyunolide F; 3-hydroxy-senkyunolide A; angeloylsenkyunolide F; senkyunolide M; 3-hydroxy-8-oxo-senkyunolide A; ligustilide; 6,7-dihydro-(6S,7R)-dihydroxyligustilide; 3a,4-dihydro-3-(3-methylbutylidene)-1(3H)-isobenzofuranone; sedanolide; and cnidilide, especially (E)-senkyunolide E, senkyunolide C, ligustilide, sedanolide, and 3-butylidenephthalide;

phytanic acid or a polyunsaturated fatty acid (also referred to herein as PUFA) in an esterified (e.g., as triglycerides or ethyl esters) or a free form, particularly an omega-3 polyunsaturated fatty acid such as eicosapentaenoic acid (5,8,11,14,17-eicosapentaenoic acid , EPA) and docosahexaenoic acid (4,7,10,13,16,19-docosahexaenoic acid, DHA), or an omega-6-polyunsaturated fatty acid such as γ-linolenic acid (6,9,12-octadecatrienoic, GLA).

In particular, the methods of the present invention are useful in inhibiting adipocyte differentiation and the associated increased in body weight observed when diabetic subjects are treated with PPARg agonists. Thus, the present compositions are particularly efficacious for the prevention and treatment of type 2 diabetes in those individuals with mild impaired glucose tolerance (IGT) and/or obesity as well as in patients with established Type 2 diabetes.

Diabetes is a chronic metabolic disease, which is caused by multiple factors and has become a major public health problem. Non-insulin-dependent diabetes mellitus (NIDDM, type 2 diabetes) is the most common form of diabetes mellitus and constitutes > 90% of the diagnosed diabetes cases in western countries. The prevalence of Type 2 diabetes mellitus is increasing rapidly, at least in part as a function of obesity. Type 2 diabetes already affects 6% of the western population and is estimated that by 2010 more than 200 million people will be affected by the disease. Type 2 diabetes mellitus is characterized by an elevated blood glucose concentration or hyperglycemia that results from abnormalities in insulin secretion and insulin action. Numerous complications of diabetes including heart disease, stroke, renal failure, retinopathy, and peripheral neuropathy contribute to the high rate of morbidity and mortality. Therefore, control of glucose homeostasis is critical for the treatment of diabetes.

There is no ideal treatment for type 2 diabetes and none of the available drugs are sufficiently efficacious to restore normal glucose levels alone or in combination therapy as the disease progresses. Diet and exercise are first-line therapies for Type 2 diabetic patients but often pharmacological intervention becomes necessary. The sulfonylureas and biguanides classes of drugs have been widely used for several decades to control blood glucose levels. Sulfonylureas are compounds that stimulate insulin release from the pancreas. However, treatment with sulfonylureas may lead to hypoglycemia and prolonged use results in side effects, particularly desensitization and/or apoptosis of the pancreatic cells resulting in decreased insulin production. Biguanides are compounds that decrease hepatic glucose output, and thus are efficacious in the treatment of hyperglycemia.

PPARg agonists have been proposed for use in the treatment of hyperglycemia and insulin resistance in patients with Type 2 diabetes. The PPARg nuclear receptor is expressed in adipose tissue and plays a pivotal role in the regulation of adipocyte differentiation. In adipose tissue, PPARg agonists promote adipocyte differentiation. In diabetic patients several weeks of TZD treatment are required to decrease plasma glucose levels. Thus, PPARg ligands that promote adipocyte differentiation may lead to increased fat accumulation and weight gain. Indeed, PPARg agonists in addition to their beneficial effects on glucose homeostasis increase fat cell differentiation and body fat accumulation in humans. Therefore, the use of these PPARg agonists is not optimal in the long-term treatment of type 2 diabetes. Obesity is highly associated with the progression of insulin resistance and any weight gain must be considered unfavorable in the treatment of type 2 diabetes if the increased body weight compromise the positive effects of the treatment.

In accordance with the present invention it has surprisingly been found that the combination of EGCG and PPARg agonists results in amelioration and/or elimination of the undesirable side effect of PPARg agonist-induced adipocyte differentiation, which leads to body fat gain. Thus, PPARg agonists such as TZD's or its pharmacologically active derivatives can be used, in combination with EGCG to treat Type 2 diabetes mellitus and to inhibit/ reduce the PPARg agonist-induced adipogenesis, while maintaining or increasing the glucose lowering effects.

The combination of EGCG and a PPARg agonist may be administered either in a single unit dosage form or by dosing each component of the combination to the patient separately in individual dosage forms administered together or sequentially. If the combination is administered as two separate compositions the administration of the two active agent occurs in a time frame over which the subject receives the benefit of the combination of both active agents.

The compositions of the invention may be in a form suitable for oral use (for example as tablets, hard or soft capsules, aqueous or oily suspensions, emulsions, dispersible powders or granules, syrups or elixirs). The compositions of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art. Thus, compositions intended for oral use may contain, for example, one or more colouring, sweetening, flavouring and/or preservative agents. EGCG may also be included in a food which may be consumed along side of a standard PPARg agonist treatment or in combination with a natural PPARg agonist such as ligustilide and analogues such as 3-Butylphthalide and 3-Butylidenephthalide, phytanic acid or a polyunsaturated fatty acids such as eicosapentaenoic acid (5,8,11,14,17-eicosapentaenoic acid , EPA) and docosahexaenoic acid (4,7,10,13,16,19-docosahexaenoic acid, DHA),

EGCG doses may be from about 0.03 to about 30 mg/kg body weight/day, more particularly from about 0.2 to about 7 mg/kg body weight/day. If EGCG is administered separately in a food or beverage, said food items may contain about 5 mg to about 500 mg EGCG per serving.

PUFA's doses may be from about 0.1 to about 60 mg/kg body weight/day, more particularly from about 0.2 to about 7 mg/kg body weight/day. If PUFA's is administered separately in a food or beverage, said food items may contain about 5 mg to about 1000 mg PUFA's per serving.

Ligustilide doses may be from about 0.01 to about 50 mg/kg body weight/day, more particularly from about 0.1 to about 20 mg/kg body weight/day. If ligustilide is administered separately in a food or beverage, said food items may contain about 5 mg to about 1000 mg ligustilide per serving.

Phytanic acid doses may be from about 0.1 to about 70 mg/kg body weight/day, more particularly from about 0.2 to about 20 mg/kg body weight/day. If Phytanic acid is administered separately in a food or beverage, said food items may contain about 5 mg to about 1000 mg Phytanic acid per serving.

The doses of the PPARg agonists, for example ciglitazone, rosiglitazone and pioglitazone will be those approved for use by regulatory bodies for example the FDA and may be found in the Orange Book published by the FDA, or otherwise proposed and known to the medical practitioner. Typical dosages are in the range of about 1 to about 1000 mg, especially about 1 mg to about 100 mg, more particularly about 1 mg to about 10 mg for an adult human of about 70 kg body weight. Alternatively, smaller doses may be used as a result of the benefits derived from the combination according to the invention.

The invention is illustrated further by the Examples which follow.

Example 1

Adipocyte differentiation

C3H10T1/2 (obtained from the American Type Culture Collection) were grown to confluence and treated with insulin alone or together with different compounds for 11 days, as shown in Table 1. After the 11-day treatment, the cells were stained with oil red O. This was followed by extraction of the stain for concentration determination. The results are shown in Table 1.

Co-treatment of C3H10T1/2 cells with ciglitazone and insulin, resulted in a higher differentiation of these cells into adipocytes than insulin alone as represented by a higher amount of oil Red O staining (Table 1). Co-treatment with insulin and EGCG at either concentration reduced the very slight adipocyte differentiation caused by insulin alone. Co-treatment with insulin, ciglitazone, and EGCG at 1×10^{-5} M resulted in a reduction of adipocyte differentiation (Table 1). EGCG at 5×10^{-5} M completely inhibited the effect of ciglitazone upon adipocyte differentiation (Table 1).

Table 1.

	Optical Density
Insulin (100 nM)	0.17 ± 0.01
Insulin (100 nM) + ciglitazone ($1x10^{-6}$ M)	0.28 ± 0.03
Insulin (100 nM) + EGCG ($1x10^{-5}$ M)	0.13 ± 0.01
Insulin (100 nM) + EGCG ($5x10^{-5}$ M)	0.08 ± 0.01
Insulin (100 nM) + ciglitazone ($1x10^{-6}$ M) + EGCG ($1x10^{-5}$ M)	0.21 ± 0.03
Insulin (100 nM) + ciglitazone ($1x10^{-6}$ M) + EGCG ($5x10^{-5}$ M)	0.08 ± 0.01

The results show that EGCG dose dependently blocked TZD-induced adipocyte differentiation. Thus, the combination of EGCG and PPARg agonist allows a pharmacological treatment that prevents progression of type 2 diabetes, while simultaneously minimizing side effects of PPARg agonists.

Example 2

Soft gelatin capsule

Soft gelatin capsules are prepared by conventional procedures using ingredients specified below:

Active ingredients: EGCG 300 mg ; Rosiglitazone 8 mg

Other ingredients: glycerol, water, gelatine, vegetable oil

Example 3

Hard gelatin capsule

Hard gelatin capsules are prepared by conventional procedures using ingredients specified below:

Active ingredients: EGCG 150 mg Rosiglitazone 8 mg

Other ingredients:

Fillers: lactose or cellulose or cellulose derivatives q.s

Lubricant: magnesium sterate if necessary (0.5%)

Example 4

Tablet

Tablets are prepared by conventional procedures using ingredients specified below:
Active ingredients: EGCG 100 mg, Pioglitazone 15 mg
Other ingredients: microcrystalline cellulose, silicon dioxide (SiO_2), magnesium stearate, crosscarmellose sodium.

Example 5

Soft Drink with 30% juice

I. A Soft Drink Compound is prepared from the following ingredients :

Juice concentrates and water soluble flavours

	[g]
1.1 Orange concentrate	
60.3 °Brix, 5.15% acidity	657.99
Lemon concentrate	
43.5 °Brix, 32.7% acidity	95.96
Orange flavour, water soluble	13.43
Apricot flavour, water soluble	6.71
Water	26.46
1.2 Color	
β-Carotene 10% CWS	0.89
Water	67.65

1.3 Acid and Antioxidant	
Ascorbic acid	4.11
Citric acid anhydrous	0.69
Water	43.18
1.4 Stabilizers	
Pectin	0.20
Sodium benzoate	2.74
Water	65.60
1.5 Oil soluble flavours	
Orange flavour, oil soluble	0.34
Orange oil distilled	0.34
1.6 Active ingredient	
EGCG	5.0

Fruit juice concentrate and water soluble flavours are mixed without incorporation of air. The color is dissolved in deionized water. Ascorbic acid and citric acid is dissolved in water. Sodium benozoate is dissolved in water. The pectin is added unter stirring and dissolved while boiling. The solution is cooled down. Orange oil and oil soluble flavours are premixed. The active ingredients as mentioned under 1.6 are dry mixed and then stirred preferably into the fruit juice concentrate mixture (1.1).

In order to prepare the soft drink compound all parts 1.1 to 1.6 are mixed together before homogenising using a Turrax and then a high-pressure homogenizer ($p_1 = 200$ bar, $p_2 = 50$ bar).

II. A Bottling Syrup is prepared from the soft drink compound from the following ingredients:

	[g]
Softdrink compound	74.50
Water	50.00
Sugar syrup 60° Brix	150.00

The ingredients of the bottling syrup are mixed together. The bottling syrup is diluted with water to 1 l of ready to drink beverage for consumption concomitant with administration of a PPARg agonist.

Example 6

Cereal Bread

Active ingredients:

EGCG and one or more additional components selected from PUFA (EPA; DHA; GLA), ligustilide, phytanic acid are incorporated in this food item

EGCG: 2-100 mg/ per serving

PUFA (EPA; DHA, GLA): 5-200 mg/ per serving

Ligustilide: 2-100 mg/ per serving

Phytanic acid: 5-200 mg/ per serving

Typical serving: 50 g

	[%]
5 cereal flour	56.8
Water	39.8
Yeast	2.3
Salt	1.1

The yeast is dissolved in a part of the water. All ingredients are mixed together to form a dough. Salt is added at the end of the kneading time. After fermentation, the dough is reworked and divided before a loaf is formed. Before baking, the surface of the loaf is brushed with water and sprinkled with flour.

Parameters:

Kneading:

Spiral kneading system	4 min 1st gear
	5 min 2nd gear
Dough proofing:	60 min
Dough temperature:	22 - 24 °C
Proofing time:	30 min

Baking:

Oven:	Dutch type oven
Baking temperature:	250/220 °C
Baking time:	50 - 60 min

What is claimed is:

1. A composition comprising a catechin found in green tea, and a peroxisome proliferator-activated receptor gamma (PPARg) agonist.

2. A composition as in claim 1 wherein the catechin is (-) epigallocatechin gallate.

3. A composition as in claim 1 or 2 wherein the PPARg agonist is a thiazolidinedione.

4. A composition as in any one of claims 1-3, wherein the thiazolidinedione is ciglitazone, rosiglitazone or pioglitazone.

5. A composition as in claim 1 wherein the PPARg agonist is a natural PPARg agonist.

6. A composition as in claim 5 wherein the PPARg agonist is a PUFA.

7. A composition as in claim 6 wherein the PUFA is eicosapentaenoic acid or docosahexaenoic acid.

8. A composition as in claim 5 wherein the PPARg agonist is ligustilide.

9. A composition as in claim 5 wherein the PPARg agonist is phytanic acid.

10. A composition as in any one of claims 2 - 9 wherein (-) epigallocatechin gallate is present in an amount sufficient to administer to a human adult a daily dosage of about 10 mg to about 2000 mg .

11. A composition as in any one of claims 1-10 which is a pharmaceutical composition.

12. A composition as in any one of claims 1-10 which is a nutritional composition.

13. The use of a catechin found in green tea and a PPARg agonist in the manufacture of a nutraceutical composition for the treatment or prevention of diabetes and/or obesity and syndrome X.

14. The use of a catechin found in green tea in the manufacture of a nutraceutical composition for concomitant consumption during treatment or prevention of diabetes and/or obesity and syndrome X by administration of a PPARg agonist.

15. The use as in claim 14 wherein the nutraceutical composition is a food or beverage or a supplement composition for a food or beverage.

16. The use as in any one of claims 13-15 wherein the catechin is (-) epigallocatechin gallate.

17. A method for the treatment or prevention of diabetes or obesity and syndrome X which comprises administering to a subject in need of such treatment an effective amount of a catechin found in green tea and of a PPARg agonist.

18. The method as in claim 17 wherein the catechin is (-) epigallocatechin gallate.

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